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Circadian oscillations in rodents

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11. Electrode labels are according to the "International 10-20 system" described by H. H. Jasper [*Electroencephalogr. Clin. Neurophysiol.* **10**, 371 (1958)].
12. The EMG electrodes were placed on responding arm only—one electrode at a third of the distance from the lateral humeral epicondyle to the styloid process of the ulna, the other electrode approximately 3.8 to 5 cm in the distal direction along the same line.
13. Trials were rejected if the sum of squared digitized values exceeded a criterion value determined by visual inspection of a large sample of EOG traces and their corresponding digital values.
14. For all subjects the nominal force levels of 25, 50, and 75 percent were set within ± 9 newtons the values 49, 98, and 147 newtons, respectively, for the nondominant hand and 68.5, 121.4, and 218 newtons for the dominant hand. The means and standard deviations of the peak force generated were determined for each subject in each of the conditions. Analysis shows that (i) the actual peak force is a monotonically increasing function of the nominal force levels (the distributions of actual force exhibit only minimal overlap), (ii) the mean peak values are consistently smaller for the nondominant hand than for the dominant one, and (iii) in the without-feedback condition response amplitude always exceeded that generated during equivalent feedback condition.
15. Analyzed by SOUPAC program BALANOVA. See *CSO volume 9 (User): Statistical Systems* (Computing Services Office, Univ. of Illinois at Urbana-Champaign, Champaign, 1974).
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3 May 1974

Circadian Oscillations in Rodents: A Systematic Increase of Their Frequency with Age

Abstract. *The circadian activity rhythms of golden hamsters and two species of deermouse, when released from a light-dark cycle of 12 hours light and 12 hours of darkness into constant darkness, had progressively shorter periods as the animals became older. A possible bearing of this fact on the aging process is briefly outlined.*

In the course of analyzing data accumulated over many years on the properties of free-running and entrained circadian pacemakers in rodents (1) we have encountered a previously unrecognized phenomenon. The period (τ) of the free-running circadian activity cycle in golden hamsters (*Mesocricetus auratus*) and two species of deermouse (*Peromyscus maniculatus* and *P. leucopus*) becomes continuously shorter as the animals age. Since these are the only species for which we have the relevant data and the effect occurs in all three, it is an interesting possibility that the phenomenon will prove to be more general. If so, it merits attention, not only as a significant variable to be controlled in the study of circadian pacemakers, but as a previously undetected aspect of the aging process.

Our experiments (2) on the circadian rhythm of activity in these rodents involved about 200 animals. The experiments included many different protocols designed primarily (i) to elucidate the stability and lability of free-running circadian pacemakers, (ii) to investigate inter- and intraspecific differences in their phase-response curves, and (iii) to relate their entrainability by light-dark cycles to these phase-response curves with the use of a model (3) based on the behavior of the circadian pacemaker in *Drosophila*.

Because of (i) the complexity in the succession of experimental treatments each animal experienced and (ii) the fact, already known and extensively further documented by the experiments themselves (1), that τ is subject to small but significant change traceable

to the oscillator's immediately previous experience, we had not thought to look in the data for systematic changes in τ attributable to the animals' ages. Indeed, the effect was initially detected in *M. auratus* and *P. maniculatus* when we sought evidence on the reproducibility of an individual animal's period (τ) in two prolonged (~ 3 months) free runs in constant darkness (DD) 9 months apart, each following an identical, and prolonged (~ 4 months) period of entrainment to the same (LD 12:12) light cycle (Fig. 1): τ was shorter when the animals were older.

The identity of the prior light cycle eliminates the possibility that this difference in τ was caused by differences in the pacemaker's previous experience (a so-called "aftereffect"). Aftereffects on τ have so far been recognized as a consequence of previous exposure to constant light, single light pulses, different photoperiods, and LD cycles with a period (T) different from 24 hours (5-7). Even the standard LD 12:12 ($T = 24$ hours) light cycle has an often significant effect in bringing τ to a value closer to 24 hours than it eventually assumes when allowed to free run for a long time (Fig. 1) (1). It follows, therefore, if one wishes to assay the effect on some variable, like age, on the frequency of a circadian rhythm (i) that the experience prior to the free run must be identical and (ii) that the effect will be better detected in a long free run when the aftereffect of that prior experience has decayed.

Figure 2 summarizes the two τ -estimates (8) obtained, 9 months apart, for each of the 17 animals in the initial comparison. In all eight hamsters and in all but one of the ten *P. maniculatus*, the τ value was smaller at age 14 to 16 months than at age 5 to 7 months.

Our data for *P. leucopus* involve eight animals which experienced a short (15 days) DD-free run (following LD 12:12) at ages 10 to 14 months, and a long (77 days) DD-free run (following LD 12:12) at ages 24 to 28 months. Seven of these eight animals also had a shorter circadian period when they were older (Fig. 2).

In hamsters an estimate of the age effect can be made over a wider range of ages than Fig. 2 covers. Figure 3 shows all the available (51) τ -estimates from long free runs (> 30 days) in hamsters of known age. The changes are small, but nevertheless statistically significant, as demonstrated by Pear-

son's correlation coefficient ($r = -.673$, $P < .001$). τ is longest in the young animals and declines systematically with age. The biggest change in τ occurs early in life. The average τ_{DD} of oldest hamsters was 24.00 hours. However, they were clearly not entrained to uncontrolled signals from the laboratory, since the activity onsets of the individuals were widely out of phase with each other and drifted slowly apart in the course of the long free runs. The data in Fig. 3 are important in another respect: they essentially eliminate the residual (though unlikely) possibility that some of the difference between the two free runs in Fig. 2 reflected the action of a circannual rhythm modulating the circadian period itself.

When all three of these rodents are placed in constant light, τ lengthens and the activity time (α) shortens (Fig. 1). Both values are a systematic function of the intensity of constant illumination (1, 9). The obvious question arises: Is α systematically related to τ when τ is changed not by illumination but by increases in age? Our data indicate, if anything, that α shortens rather than lengthens when τ shortens with age (1).

While there have been studies on the ontogeny of circadian rhythmicity, they have been mostly concerned with the time in development at which a circadian pacemaker is differentiated (10), although the amount of activity as well as its temporal distribution in a light-

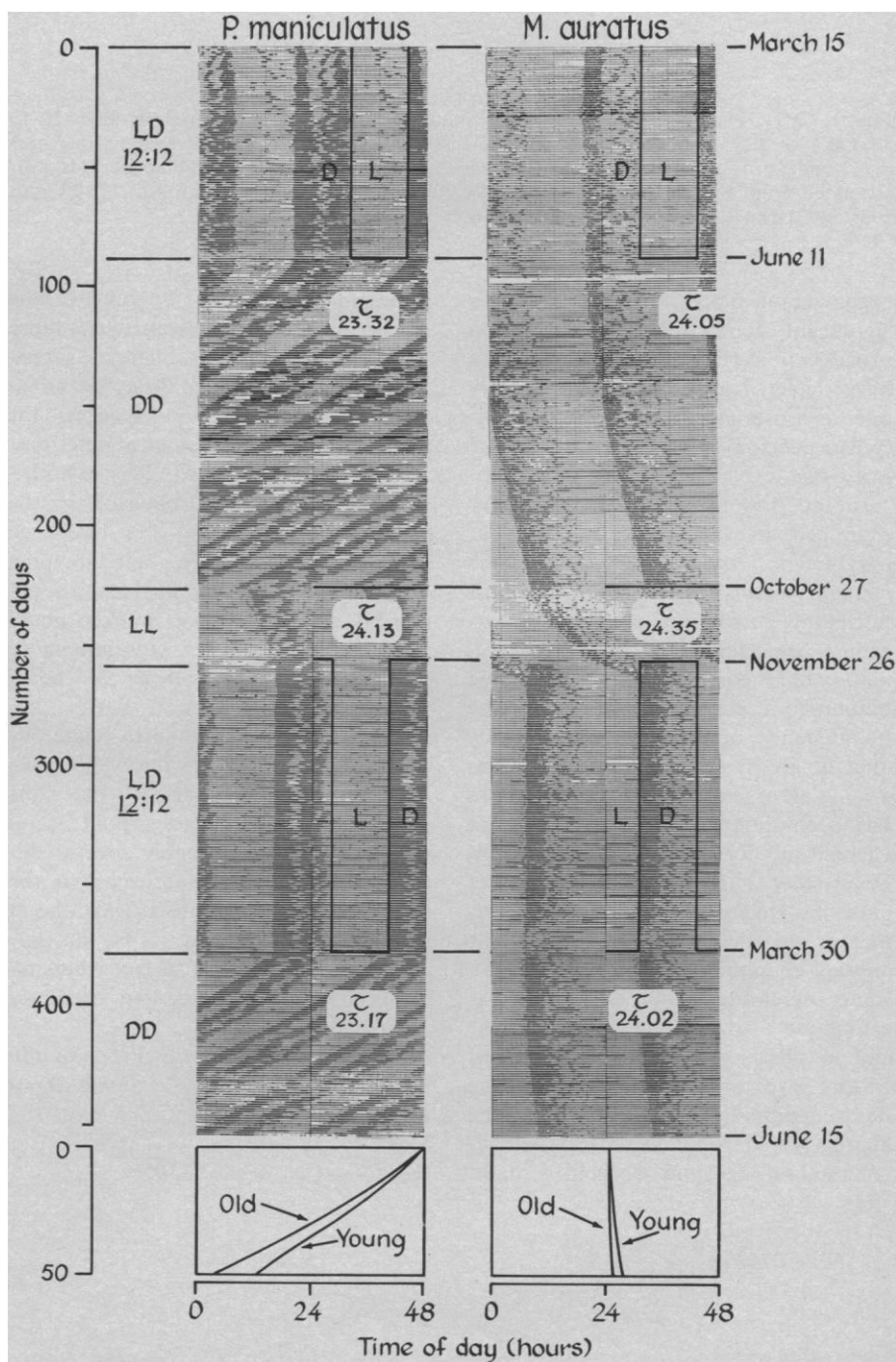
dark cycle has been reported to change with age (11). To our knowledge, there has been no previous observation that any property (for example, frequency) of a circadian pacemaker (as distinct from the pattern of the rhythm it drives) is a function of developmental time.

There is no obvious functional meaning to the systematic increase of circadian frequency with age. The only prediction the observation invites is that, unless the shape of the pacemaker's phase-response curve also changes systematically with age, the phase relation (ψ) between the pacemaker and the environmental cycle driving it will

become increasingly less negative (more positive) with age. But this is unlikely to be either a large effect or, in itself, functionally important. Only in *P. maniculatus* is the change in τ large enough to predict that in nature the first animals to become active in the evening will be the older ones, and the last to disappear in their holes in the morning will be juveniles.

There are, however, two important implications of our observation. First, age (or "developmental time") cannot now be ignored in the analysis of circadian systems. The second is the possibility that other changes in circadian

Fig. 1. Activity records of a deer mouse (*Peromyscus maniculatus*; 1512, born 11 February 1965) and a golden hamster (*Mesocricetus auratus*; 1491, born 12 December 1964). Data for successive days are placed below each other, and the entire record is "double-plotted" to facilitate estimation of the periodicity of activity onsets and cutoffs. Light treatments indicated in the left margin and in the right half of each record include: 12 hours of light and 12 hours of darkness per day (LD 12:12); constant darkness (DD); constant light (LL). Numbers in the right half of the figures are estimated τ values for days 20 to 30 of the free runs. They are among the τ estimates in Fig. 2. The onset of activity of the deer mouse shifted 34.1 hours forward in days 0 to 50 of the first DD run and 40.8 hours forward in days 0 to 50 of the second DD run. In the same periods, the onset of activity in the hamster shifted backward 2.4 hours and 1.1 hours, respectively. The rates of shifting in the two runs are compared in the bottom panels; the activity onsets of the young and old animal (in each case) have been made synchronous initially to facilitate the comparison.



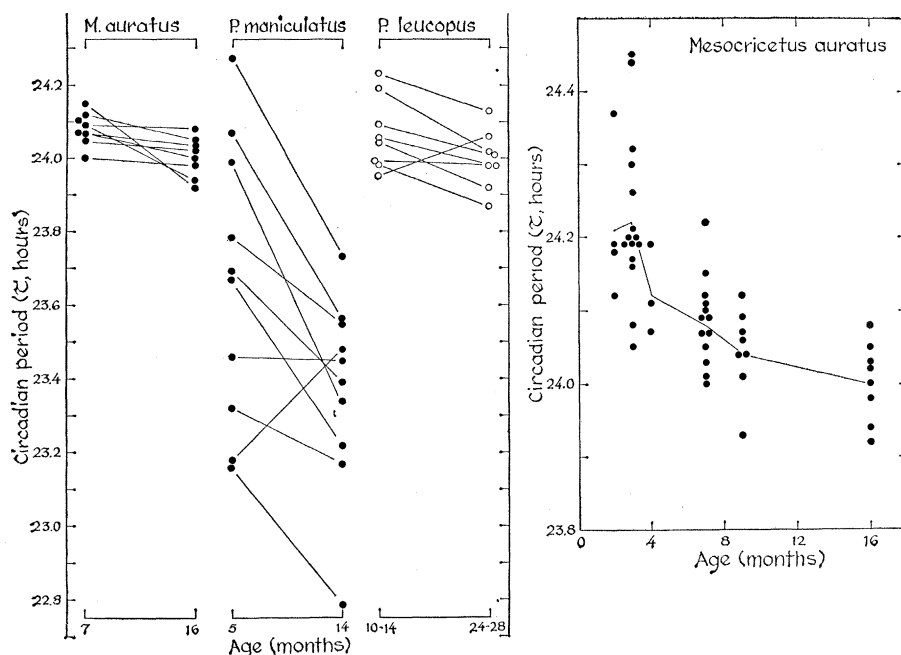


Fig. 2 (left). Circadian periods (τ), estimated from the activity onset in days 20 to 30 (●) or days 0 to 10 (○), after release from LD 12:12 into constant darkness in 25 rodents tested twice in their lifetimes. Fig. 3 (right). Circadian periods (τ), estimated from the activity onsets in days 20 to 30 after release from LD 12:12 into constant darkness in hamsters, plotted as a function of their age.

organization, more generally, may be a significant concomitant of the aging process. It should be stressed that we have detected an aging effect on only one measure (frequency) of one circadian pacemaker (that driving the activity/rest cycle); it is the only measure and the only pacemaker we have examined in this respect. Do other parameters, and other pacemakers change? In this context quite the most intriguing possibilities concern the circadian organization of the whole animal, which comprise more than one autonomous pacemaker (5, 12). The maintenance of a common frequency and a given set of phase relations among an organism's circadian rhythms has been thought to be an important element in "normal" physiological well-being (6, 13). If, as could well be the case, the frequencies of different pacemakers in the organism, and their mutual coupling, change differently—some increasing, some decreasing—as a function of developmental time, their mutual phase relations would change. In that case, a systematic change in the daily temporal organization of the animal would result as at least a concomitant of age and it could also, in

principle, be a cause of some of the physiological effects one attributes more simply to "age." Thus, there is a testable hypothesis that a decay of circadian organization is involved in the preprogrammed physiological deterioration that limits life-span. This hypothesis is already rendered plausible by the fact that longevity in flies has been shown to decline when their circadian organization is maintained in an abnormal state by major weekly phase shifts or by continuous entrainment to frequencies different from 24 hours (14).

The obvious temptation to relate our present observations to the well-known human subjective experience that time passes faster as one ages should be resisted. There are probably several different physiological substrates to the subjective sense of time, at least one of which (15) is known to be strongly temperature-dependent, a fact which almost eliminates a circadian oscillator as the clock involved.

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